

INTEGRATING BIOAVAILABILITY APPROACHES INTO WASTE ROCK EVALUATIONS¹

James Ranville², Eric Blumenstein, Marty Adams, LaDonna Choate, Kathleen Smith, and Thomas Wildeman

Abstract: The presence of toxic metals in soils affected by mining, industry, agriculture and urbanization, presents problems to human health, the establishment and maintenance of plant and animal habitats, and the rehabilitation of affected areas. A key to managing these problems is predicting the fraction of metal in a given soil that will be biologically labile, and potentially harmful ('bioavailable'). The molecular form of metals and metalloids, particularly the uncomplexed (free) form, controls their bioavailability and toxicity in solution. One computational approach for determining bioavailability, the biotic ligand model (BLM), takes into account not only metal complexation by ligands in solution, but also competitive binding of hardness cations (Ca^{2+} , Mg^{2+}) and metal ions to biological receptor sites. The more direct approach to assess bioavailability is to explicitly measure the response of an organism to a contaminant. A number of microbial enzyme tests have been developed to assess the impact of pollution in a rapid and procedurally simple way. These different approaches in making bioavailability predictions may have value in setting land-use priorities, remediation goals, and habitat reclamation strategies.

Additional Key Words: enzyme bioassay, biotic ligand model, metal contamination, mine wastes, contaminated soils and sediments, toxicity testing

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² James F. Ranville is an associate professor in the Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO, 80401. Eric Blumenstein and Marty Adams are MS students in the Division of Environmental Science and Engineering, Colorado School of Mines, Golden, CO, 80401. LaDonna M. Choate is a Mendenhall Postdoctoral Fellow at the United States Geological Survey. Kathleen Smith is a research chemist at the United States Geological Survey. Thomas Wildeman is a Professor Emeritus in the Department of Chemistry and Geochemistry, Colorado School of Mines, Golden, CO, 80401.

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Introduction

Abandoned and inactive mining operations have generated, and continue to generate, vast amounts of mining waste throughout the United States. Mine waste represents any material that has been produced and left behind in any part of the mining process, including but not limited to: waste and development rock, mine tailings piles, sedimentation ponds, and contaminated soils/stream sediments (Durkin and Herrmann, 1994). Mine wastes often contain metals and high acidity, which may be toxic to the surrounding biota. In the case of metals, their mere presence does not necessarily imply a toxic condition will result. The mineralogical form of the metal in the waste as well as the environmental conditions into which it is released influence whether it is “bioavailable”, that is, whether the metal is present in a form that can be taken up by an organism. The United States Environmental Protection Agency (USEPA) defines bioavailability as “A measure of the physicochemical access that a toxicant has to the biological processes of an organism” (USEPA 2004a). The less bioavailable a toxicant, the less its toxic effect on an organism will be. The three major components that determine bioavailability are: concentration of the metal present in the system, competition between the heavy metal ions and other competing ions for binding sites on the biota, and the complexation of metallic ions with inorganic and organic compounds. These concepts are illustrated in Fig. 1 and are described below:

1. Concentration – the amount of total heavy metals and heavy metal ions present in the aqueous phase of the system. The higher the concentration of a metal present, the more toxicity there will be. This is controlled by “a” in Fig. 1.
2. Competition – other cations competing (shown as “d”) with the metal ions for binding sites on the biota “c”. The most important cations competing for binding sites are those of hardness (Ca^{+2} , Mg^{+2} , and Na^{+}); others include protons (H^{+}) and potassium (K^{+}).
3. Complexation – metallic ions complexing (b.) with inorganic and organic compounds that are present in the aqueous portion of the system “c”. Inorganic complexation in freshwater includes ions making up alkalinity (HCO_3^- , CO_3^{-2}) among others. Organic complexation occurs with natural organic matter (NOM), measured as either total organic carbon (TOC) or dissolved organic carbon (DOC).

Bioavailability is also controlled by factors that are involved in the transport of metals within the organism (e). In cases of aquatic toxicity, the process is somewhat simplified in that the site of toxic action is also the site of uptake on the gill surface of aquatic species.

Because of the abundance of abandoned mine sites, the potential toxicity stemming from the mine waste, and limited available resources for abandoned mine remediation, it is important to develop a procedure to rank sites in order from those that have the highest potential for adverse environmental and human health impacts to those that have the lowest potential. Therefore, a decision tree has been made for the purpose of ranking these abandoned mine sites throughout the state of Colorado and the entire Western United States (Wildeman et al., 2003). This decision tree, illustrated in Fig. 2, evaluates a waste site using both physical and chemical characteristics. In this paper we will focus on the chemical characterization only. The first step

in the chemical characterization is to determine the pH of the mine waste pile leachate using a deionized water extraction (paste pH). If the pH is less than 5, the pile should be considered toxic and a toxicity characteristic leaching procedure (TCLP) using the USEPA Method 1311 (USEPA, 1992) should be run. If the pH is close to or above 5, the toxicity is uncertain and further testing needs to be done. In that case we propose that a simple bioavailability test should be conducted to confirm or deny toxicity. A complementary approach is to examine the leachate water chemistry using a toxicity/metal speciation model to determine what organisms will be vulnerable in the surrounding environment.

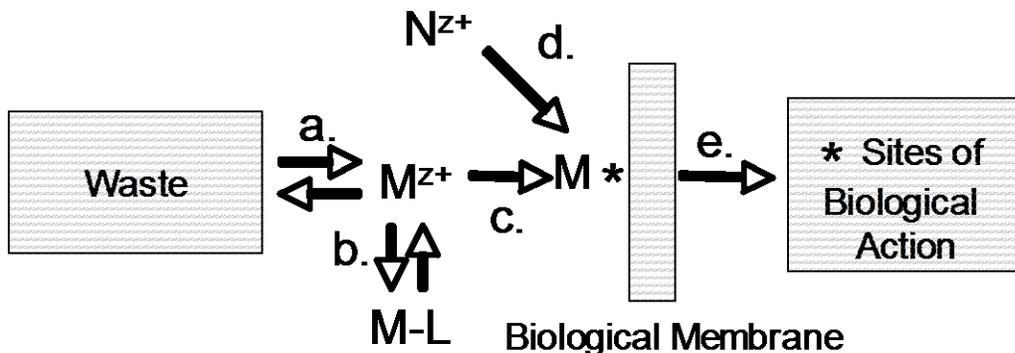


Figure 1. Major processes controlling the bioavailability of metals associated with mine waste include: a. association (precipitation/sorption) or release (dissolution/desorption), b. complexation in solution (dissolved organic carbon and carbonate/hydroxide ligands), c. binding at the biological site of uptake, d. competitive uptake of competing cations (hardness, pH, sodium), e. internal transfer within the organism.

The Decision Tree

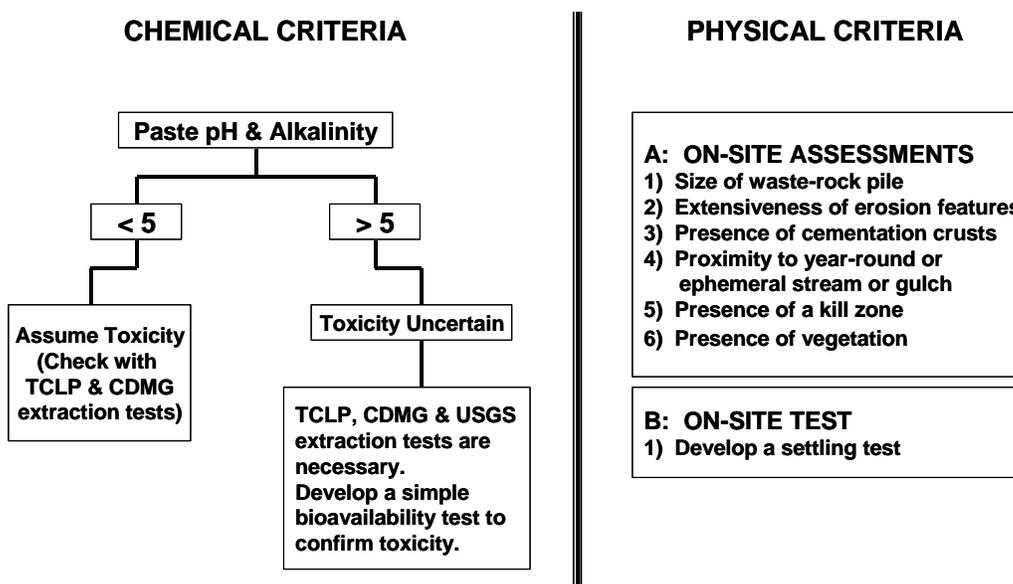


Figure 2. Mine site evaluation scheme incorporating bioavailability assessments.

The Biotic Ligand Model (BLM) is a toxicity model that accounts for the effects of water chemistry (hardness, alkalinity, pH, dissolved organic matter) based on the speciation of metals and their bioavailability to aquatic biota (Di Toro et al., 2001; Santore et al., 2001; Gorsuch et al., 2002; Niyogi and Wood, 2004). It is used to develop site-specific water-quality criteria and to build a framework for the regulation of metals. Given site-specific water chemistry, a chosen metal, and a chosen organism, the BLM predicts the LC₅₀ which is the metal concentration that results in the death of 50% of the exposed test organisms. The BLM approach can be used in conjunction with the waste decision tree to predict the extent of environmental impact of a given waste material.

Although the chemical analysis of waste rock leachates provides key information about the potential toxicity of a waste, several other factors may mitigate the degree to which released metals pose a hazard. Adding a direct measure of toxicity to the waste decision tree improves our ability to characterize wastes. Several enzyme and microbial assays have been proposed for assessing the toxicity of environmental samples (Bitton and Dutka, 1986; Bitton and Koopman, 1992; Bitton and Morel, 1998). Most of the proposed toxicity tests measure the general toxicity of a sample that can arise from organic or inorganic toxicants. The MetPLATE™ enzymatic toxicity assay test kit focuses on the specific determination of heavy metal toxicity (Bitton et al., 1994; Nelson and Moline, 1998). Because the toxic components of mining influenced waters (MIW) are most often heavy metals, the MetPLATE™ test kit is an appealing enzymatic bioassay for our application. A major question regarding the use of microbial enzyme bioassays is whether they respond to changes in water chemistry in a manner similar to that predicted by the BLM for aquatic organisms such as *ceriodaphnia dubia*.

In this paper, we describe and evaluate different methods for determining bioavailability with respect to aquatic toxicity. From these preliminary studies we hope that the utility of incorporating bioavailability assessments into the evaluation of waste rock piles is demonstrated.

Methods

Water Samples

Two types of water samples were investigated in this study. To test the BLM, water samples were collected from the North Fork of Clear Creek, located near Golden, Colorado. A site located downstream of the metal-rich water inputs in the BlackHawk/Central City area was selected. A site located upstream of the mining sites was also sampled in order to have dilution water that, although having a similar hardness, alkalinity and DOC levels, was low in Cu and Zn. Grab samples were collected in polyethylene sample bottles and filtered in the field using 0.45 micron cellulose nitrate filters (Sartorius, Inc.). Samples for chemical analysis were acidified to pH < 2 using HNO₃. Samples for testing the effect of water chemistry on MetPLATE™ were prepared in the laboratory using reagent-grade chemicals. All samples used in toxicity testing were refrigerated at 4°C between collection/preparation and the time of testing. Alkalinity was determined by titration to the bromocresol green endpoint (pH about 4.5) using an alkalinity kit (HACH). Sample pH was determined using a portable pH meter (Orion) with values considered accurate to ± 0.1 units.

Chemical Analyses.

All water samples, both laboratory-prepared and field-collected were analyzed for elemental concentrations using Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

(Lamothe et al., 2002) at the Colorado School of Mines (CSM). The samples were acidified with HNO₃ to less than 2, then analyzed on a Perkin Elmer Optima 3000 ICP-AES for 31 elements including two of greatest importance for this study; Cu and Zn. Copper and Zn were chosen because they are the two metals that are thought to be toxic to the biota in the systems we are focusing on. During the ICP-AES analysis, an internal standard of scandium is used to correct for variations in sample uptake and plasma conditions. Concentration check standards are analyzed in the beginning and after every 20 samples to monitor the stability of all analytical conditions. The relative standard deviation of the water sample results is about ±5% for concentrations that are greater than 10 times the limit of detection.

Dissolved organic carbon (DOC) was analyzed by a UV-catalyzed persulfate oxidation method on samples acidified to pH <4 with H₃PO₄, using a Sievers TOC 800 Turbo instrument at CSM.

Biotic Ligand Modeling.

The BLM was developed from a combination of biological and toxicological measurements (Pagenkopf, 1983 ; Playle, 1998) and chemical speciation and complexation models (Tipping, 1998). The BLM incorporates the speciation computation and relates this to observed metal toxicity, presented as the LC₅₀. The latest version of the BLM (v 2.1.2), as developed by HydroQual (Di Toro et al., 2001; Santore et al., 2001; Santore et al., 2002), is available for download at http://www.hydroqual.com/wr_blm.html. Inputs into the BLM program include: temperature, pH, concentration of metal under evaluation, DOC, percent humic acid, Ca, Mg, Na, K, SO₄⁻², Cl⁻, alkalinity, and sulfide concentrations. These water chemistry components define the inorganic and organic metal interactions that control the concentration of metal present in the free ion form. This concept is based on the free ion activity model (FIAM) (DiToro et l., 2001). The BLM computation for metal interaction with organic carbon is based on the Windermere humic aqueous model WHAM 6 (Tipping, 1998) , and all inorganic complexation reactions are simulated by the chemical equilibrium speciation in soils and solutions model (CHESS; Santore and Driscoll, 1995). The BLM relates mortality to a critical concentration of metal bound to the biotic ligand, defined as the lethal accumulation (LA50) that results in 50 % mortality (Di Toro et al. 2001). The BLM computes the speciation of a metal based on the water quality conditions and the amount of metal that has to be present to cause toxicity as a result of the metal's affinity for binding sites on the gill.

Ceriodaphnia dubia tests.

The U.S. Environmental Protection Agency standard operating procedure was used for the *ceriodaphnia dubia* toxicity tests (U.S. Environmental Protection Agency, 2002). This procedure involves a 48-hour static test during which time the samples are held at 20°C ± 1°C. Each day of the test consists of 16 hours of light and 8 hours of dark to simulate a diurnal cycle. In our tests, each individual test chamber consisted of a 30-mL plastic cup containing 15 mL of test solution. Five test organisms were used per test chamber, and each of the organisms were less than 24 hours old, cultured in moderately hard reconstituted water, and fed for two hours prior to transfer into the test chambers. After 48 hours, each test chamber was examined to determine the mortality of the test organisms. The reported value is the lethal concentration at which 50% of the test organisms died (LC₅₀). The results of the test were considered valid if 90% of the organisms in the control survived.

In this study, Cu was added to a water sample collected from the North Fork of Clear Creek at a site downstream of the mined zone. This spiked sample was then diluted with a water

sample collected from an uncontaminated upstream site. A range of five Cu concentrations and a control (water collected from an upstream source). The range of desired Cu concentrations was determined by running the BLM to obtain a predicted LC₅₀ for Cu in the simulated test-water types and then bracketing that predicted LC₅₀ with a range of Cu concentrations. The same procedure was employed with Zn.

MetPLATE™ Toxicity Assay.

The MetPLATE™ kit (University of Florida, Gainesville, Florida) includes freeze-dried *E. coli* (“Bacterial Reagent”), a chromogenic substrate that is hydrolyzed by the bacteria (“substrate”), moderately hard water (“Diluent”), phosphate buffered enzyme substrate (“Buffer”), and one 96-well microplate. The freeze-dried bacterial reagent is rehydrated into 5.0 mL of diluent and is mixed thoroughly by vortexing until a uniform suspension is obtained. A volume of 0.1 mL of rehydrated bacterial reagent is then added to 0.9 ml of sample or a dilution thereof. Typically 5-6 serial 50:50 dilutions are made. The mixture is then vortexed and incubated at 35°C for 60 minutes. At the end of the 60-minute exposure period a 0.2 ml aliquot of the suspension is dispensed into a well of the assay microplate. Then, 0.1 ml of the chromogenic substrate is added to each well. Upon mixing in each of the wells, the microplate is incubated an additional 60 to 90 minutes at 35°C for color development. The intensity of the resulting purple color of the hydrolyzed chromogenic substrate gives an indication of enzyme (β -galactosidase) activity and is inversely proportional to the toxicity of the sample, that is, a darker color indicates lower toxicity. Absorbance is measured at 575 nanometers (nm) using a 96-well microplate reader (PowerwaveX 340). All toxicity tests and samples are run in triplicate (Bitton et al., 1994). An example of a typical MetPLATE™ assay after incubation that is measured in the 96-well spectrophotometer can be seen in Fig. 3.

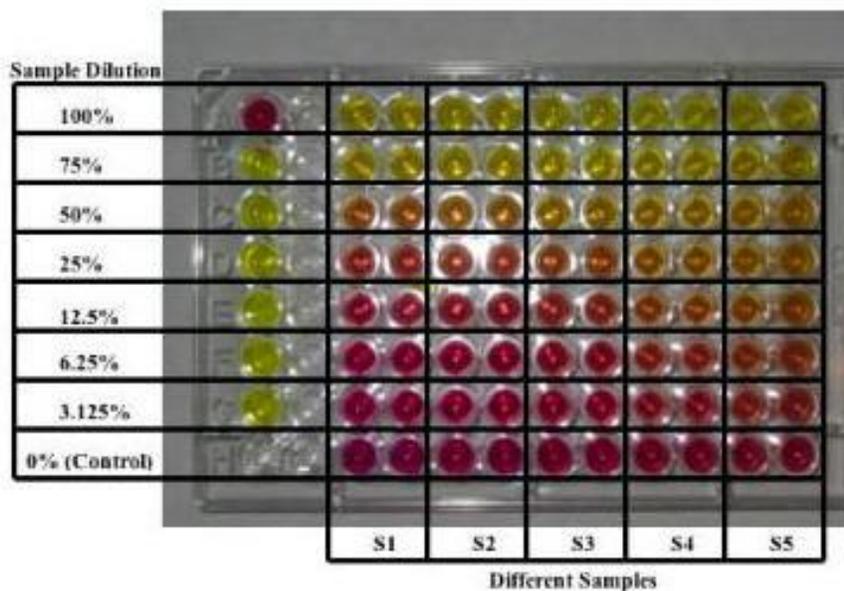


Figure 3. The MetPlate microbial enzyme bioassay showing the effect of dilution of a copper – containing solution on the colorimetric change in the added chromophore. Decreasing toxicity, which results from dilution, corresponds to an increase in absorbance at 560 nm.

Results and Discussion

We have applied both the BLM predictions and enzyme bioassays to several systems including the Tip Top Mine and the BlackHawk-Central City Superfund site, both located in Colorado. In the Tip Top Mine study, both waste rock leachates and the impacted stream (water and sediments) were examined (Moehl et al.,2006). In the Clear Creek system we have up to this point focused on the streamwater. In this paper we will focus on the lessons learned from the Clear Creek system and from the MetPlate laboratory studies.

Currently the effect of water chemistry on metal toxicity is only partially accounted for in water quality regulations. This is done by use of hardness-based equations to compute the water quality criteria for a stream. There are equations for Cd, Cr⁺³, Cu, Pb, Ni, Ag, and Zn, because those are metals of toxicological concern in water for both human health and other biota. The calculations and equations were updated in 1995 (USEPA 1995). The criterion maximum concentration (CMC) is the maximum amount of dissolved metal allowed in a freshwater, which is computed from:

$$\text{CMC (dissolved)} = \exp\{m_A [\ln(\text{hardness})] + b_A\} (\text{CF}) \quad (1)$$

where the parameters for the CMC equation are given as (USEPA, 1999):

Copper: $m_A = 0.9422$, $b_A = -1.700$, $\text{CF} = 0.960$

Zinc: $m_A = 0.8473$, $b_A = 0.884$, $\text{CF} = 0.978$

The CMC was developed to assure that water quality criteria are protective of most aquatic species, without being overly restrictive when higher hardness occurs. In this study the CMC for Cu and Zn are used as relative measures of toxicity in order to relate the effect of water chemistry predicted by this approach to the BLM prediction of toxicity, based on the LC50 of copper and zinc for *ceriodaphnia dubia*.

The CMC approach, while an improvement over using total acid recoverable metal concentration does not include all aspects of water chemistry that influence metal toxicity. The most important example of an aspect that is not covered is the role of DOC as a complexer of some metals.

To evaluate the improvement in predictions of metal toxicity, over the CMC approach, the BLM was used to examine a site on Clear Creek, Colorado in 2005. Seasonal changes in hydrology caused significant variations in not only the levels of toxic metals, but also in the constituents responsible for toxicity mitigation (hardness, DOC, alkalinity, and pH). One of the periods of greatest variation was noted during spring runoff when snowmelt can greatly affect water composition. Snowmelt from the upper reaches of the watershed, outside the mined areas, can cause significant dilution of the stream water. Recharge in the vicinity of the mining area has an opposite effect in that snowmelt can “flush” water from the mines thus increasing metal loads to the stream. The dissolved organic carbon concentration (DOC) can also be elevated during this time as a result of leaching from the surrounding soils. Because of the dynamic nature of the water chemistry during spring runoff, we chose to sample the North Fork of Clear Creek in order to perform and evaluate toxicity tests. The results of laboratory toxicity tests were compared to BLM predictions as well as to the current hardness-based acute water quality criteria.

We sampled the site on April 14th and May 11th, 2005. The first date is considered pre-runoff while the second sampling date is on the rising limb of the hydrograph. The results of the water analysis are presented in Table 1. As discharge increased, the concentrations of Ca, Mg, Na, and K all decreased. Interestingly the concentration of Cu and Zn increased, suggesting a flushing of the mines occurred as runoff progressed. An increase in DOC was observed. Both pH and alkalinity remained nearly constant. Because the hardness was lower in May than April, the criteria maximum concentration for Zn and Cu, as computed by equation 1, was lower in May, as shown in Table 2. The measured concentrations of Zn were above these values for both dates whereas the measured Cu concentration only exceeded the CMC in May. These results suggest that metal toxicity, due in part to the lowering of the hardness, could be an issue even during spring runoff.

Table 1: North Fork water chemistry data.

	Temp	pH	Cu	Zn	DOC	Ca	Mg	Na	K	SO ₄	Alkalinity
	°C		ug/L	ug/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
4/14/05	10.9	7.8	9.1	259	1.6	26.3	8.4	25.3	3.2	26.1	16.8
5/11/05	5.3	7.6	24.3	357	4.0	11.7	3.8	7.96	1.6	72.7	19.2

Table 2: EPA recommended acute water quality criteria: Criteria Maximum Concentration (CMC) calculated using data from Table 1.

	Computed Hardness	CMC Copper	CMC Zinc
	mg/L CaCO ₃	(ug/L)	(ug/L)
4/14/05	100.2	13.5	117
5/11/05	44.8	6.3	59.3

Although the CMC for copper and zinc are not equivalent to the LC50 for *ceriodaphnia dubia*, the effect of water chemistry on each can be used for comparative proposes. For Cu the interpretation of the effect of changing water chemistry on toxicity is very different for the BLM and CMC approaches. In contrast to the hardness-based prediction, the BLM predicted a higher LC50 for Cu in the May sample as compared to the April sample (Table 3). Instead of a 2-fold lowering of the CMC (from 13.5 to 6.3), the BLM suggests that the criteria might be raised by a factor of 1.5 and still be protective. The BLM result is in general agreement with the toxicity tests, for which an LC50 of greater than 35 microgram per liter was observed in May as compared to 16.5 in April. For Zn the influence of water chemistry on the experimental and BLM-computed LC50's is in agreement with the hardness-based approach. This may be consistent with the lesser amount of NOM complexation of Zn as compared to Cu. Both the BLM and the toxicity tests suggested that the Cu and Zn levels were non-toxic, as shown by Toxic Units (TU) of less than one for both metals at both dates. Thus a very different interpretation is made if the CMC versus BLM approach is used. Although the BLM LC50

predictions and the measured LC50s are not in perfect agreement, they are more consistent between dates than that predicted by the CMC approach.

Table 3: Measured Cu and Zn LC50, BLM computed LC50 and computed toxic units for the Clear Creek sample. (Toxic Units (TU) = [measured metal concentration] / [LC₅₀])

Date	Cu				Zn			
	BLM LC ₅₀ (ug/L)	BLM Toxic Units	Measured LC ₅₀ (ug/L)	Measured Toxic Units	BLM LC ₅₀ (ug/L)	BLM Toxic Units	Measured LC ₅₀ (ug/L)	Measured Toxic Units
4/14/05	28.01	0.32	16.5	0.55	1117	0.23	595	0.44
5/11/05	46.6	0.52	> 35	<0.7	880	0.41	369	0.97

The *ceriodaphnia dubia* method is a regulatory test and is useful for verifying the BLM computations, but is difficult to perform on a routine basis. The use of enzyme bioassays as a rapid screening tool is very appealing due to its ease of use. If these tests are to be of value then they must capture the effects of water chemistry on toxicity similar to those seen for the aquatic organisms used in the test. As part of our evaluation of the MetPlate test we have examined the roles of hardness and alkalinity on the results. We performed Cu and Zn toxicity tests in five waters of varying hardness and alkalinity prepared using standard USEPA methods. After normalizing the data to the blank controls in each water, the degree of inhibition of the enzyme activity of the bacteria by the metals could be determined. The results of these tests are shown in Fig. 4 and 5. As seen in the figures, increasing hardness and alkalinity affect both Cu and Zn toxicity to *e. coli* in the MetPlate test. The highest hardness shows a greater affect on Zn than on Cu. These results, while still preliminary are encouraging in that it appears that the affects of water chemistry on Cu and Zn toxicity to *ceriodaphnia dubia* are qualitatively captured in the MetPlate™ assays.

Summary

We have just begun to examine the utility of the BLM and enzyme bioassays in the evaluation of mining influenced waters, soils, and sediments. The BLM appears to improve our prediction of metal toxicity in Clear Creek by capturing the role of DOC on copper toxicity while also accounting for hardness-based effects. The enzyme bioassays are a much simpler approach than traditional toxicity tests and appear to capture effects of water chemistry on toxicity similar to that seen for *ceriodaphnia dubia*. Further work on assessing bioavailability will continue to improve our understanding of the true ecological effects of metals in mining impacted areas.

Copper Concentration vs. MetPLATE % Inhibition

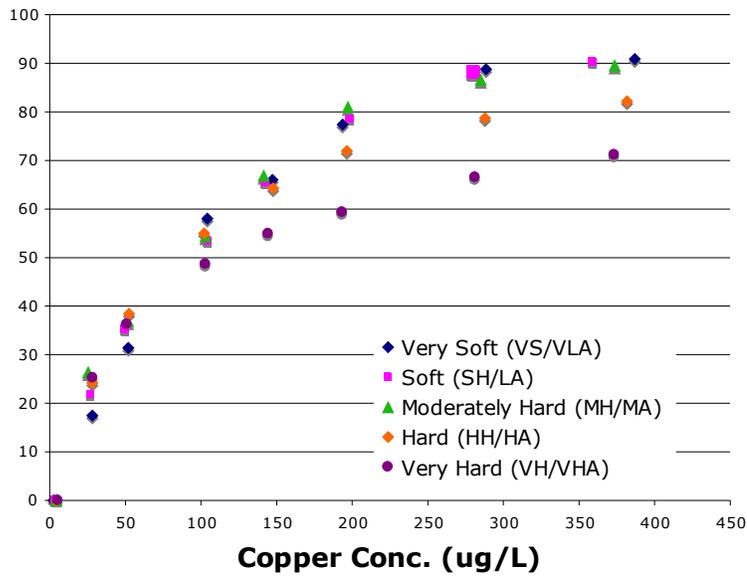


Figure 4. Influence of water composition on the toxicity (enzyme inhibition) of copper to *E. coli* in the MetPlate bioassay.

Zinc Concentration vs. MetPLATE % Inhibition

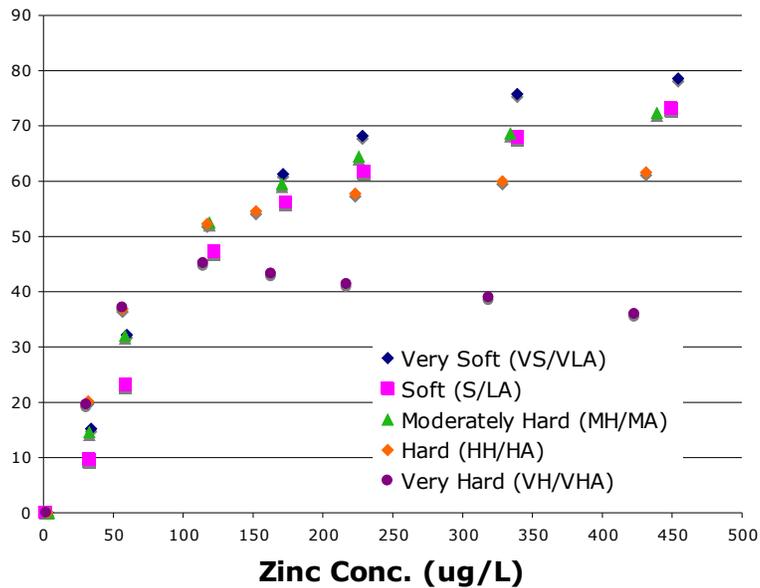


Figure 5. Influence of water composition on the toxicity (enzyme inhibition) of zinc to *E. coli* in the MetPlate bioassay.

Literature Cited

- Bitton, G. and B. J. Dutka. 1986. Toxicity Testing Using Microorganisms. Vol. 1. Boca Raton, FL: CRC Press.
- Bitton, G., K. Jung, B. Koopman. 1994. Evaluation of a Microplate Assay Specific for Heavy Metal Toxicity. Arch. Environ. Contam. Toxicol. v. 27, p. 25-28. <http://dx.doi.org/10.1007/BF00203883>.
- Bitton, G. and B. Koopman. 1992. Bacterial and Enzymatic Bioassays for Toxicity Testing in the Environment. Rev. Environ. Contam. Toxicol. v. 125, p. 1-22 http://dx.doi.org/10.1007/978-1-4612-2890-5_1
- Bitton, G. and J. L. Morel. 1998. Enzyme Assays for the Detection of Heavy Metal Toxicity. In: Wells, P. G., K. Lee, and C. Blaise (ed.). Microscale Aquatic Toxicology: Advances, Techniques and Practice. Boca Raton, FL: CRC Press.
- Di Toro, D.M, Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., and Santore, R.C. 2001. Biotic ligand model of the acute toxicity of metals 1. Technical basis. Environmental Toxicology and Chemistry, v. 20, p. 2383-2396. <http://dx.doi.org/10.1002/etc.5620201034>.
- Durkin, T. V., Herrmann, J. G. 1994. Focusing on the Problem of Mining Wastes: An Introduction to Acid Mine Drainage. EPA/625/R-97/007 "Managing Environmental Problems at Inactive and Abandoned Mine Sites." 1994. Anaconda, MT, Denver, CO, Sacramento, CA.
- Gorsuch, J.W., Janssen, C.R., Lee, C.M., and Reiley, M.C. (eds.). 2002. Special issue: The biotic ligand model for metals—Current research, future directions, regulatory implications. *Comparative Biochemistry and Physiology, Part C Toxicology and Pharmacology*, v. 133C, no. 1-2, 343 pp.
- Lamothe, P.J., Meier, A.L., and Wilson, S.A. 2002. The determination of forty-four elements in aqueous samples by inductively coupled plasma-mass spectrometry. p. H1-H11. In: J.E. Taggart Jr., ed., Analytical methods for chemical analysis of geologic and other materials. U.S. Geological Survey Open-File Report 02-223. (Available online at <http://pubs.usgs.gov/of/2002/ofr-02-0223/>; accessed October 2, 2005.)
- Moehle, J., Ranville, J. F., Choate, L.M., Wildeman, T.R., and Ross, P. 2005. Leachate and enzyme boassay toxicity assessments at the Tip Top mine., a marginally impacted site, Proceedings America Society of Mining and Reclamation, 2005 pp 737-749 <http://dx.doi.org/10.21000/JASMR050107379>
- Nelson, S. M. and R. A. Roline. 1998. Evaluation of the Sensitivity of Rapid Toxicity Tests Relative to Daphnid Acute Lethality Tests. Bull. Environ. Contam. Toxicol., v. 60, p. 292-299. <http://dx.doi.org/10.1007/s001289900624>.
- Niyogi, S., and Wood, C.M. 2004. Biotic ligand model, a flexible tool for developing site-specific water quality guidelines for metals. Environmental Science and Technology, v. 38, no. 23, p. 6177-6192. <http://dx.doi.org/10.1021/es0496524>.
- Pagenkopf, G.K. 1983. Gill surface interaction model for trace-metal toxicity to fishes: Role of complexation, pH, and water hardness. Environmental Science and Technology, v. 17, p. 342-347. <http://dx.doi.org/10.1021/es00112a007>.

- Playle, R. C. 1998. Modeling metal interactions at fish gills. *Sci. Total Environ.*, 219, p. 147-163. [http://dx.doi.org/10.1016/S0048-9697\(98\)00232-0](http://dx.doi.org/10.1016/S0048-9697(98)00232-0).
- Santore, R.C., Di Toro, D.M., Paquin, P.R., Allen, H.E., and Meyer, J.S. 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and *Daphnia*. *Environmental Toxicology and Chemistry*, v. 20, no. 10, p. 2397-2402 <http://dx.doi.org/10.1002/etc.5620201035>
- Santore, R.C., Mathew, R., Paquin, P.R., Di Toro, D. 2002. "Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow and *Daphnia magna*." *Comparative Biochemistry and Physiology. C. Pharmacology, Toxicology and Endocrinology.*, Vol. 133, pp. 271–285. [http://dx.doi.org/10.1016/S1532-0456\(02\)00106-05](http://dx.doi.org/10.1016/S1532-0456(02)00106-05).
- Santore, R.C. and Driscoll, C.T. 1995. The CHESS model for calculating chemical equilibria in soils and solutions. p. 357-375. *In*: S. A. Loeppert and S. Goldberg (ed.), *Chemical Equilibrium and Reaction Models*. Soil Science Society of America Special Publication. Vol. 42. Madison, WI, American Society of Agronomy.
- Tipping, E. M. 1998. Humic Ion-Binding Model VI: An Improved Description of the Interactions of Protons and Metal Ions with Humic Substances. *Aquatic Geochemistry*, 4, p. 3-47. <http://dx.doi.org/10.1023/A:1009627214459>
- U.S. Environmental Protection Agency. 1992. Method 1311 Toxicity Characteristic Leaching Procedure. (Available online at www.epa.gov/epaoswer/hazwaste/test/pdfs/1311.pdf).
- U.S. Environmental Protection Agency. 1999. National Recommended Water Quality Criteria-Correction. US EPA. Washington D.C. EPA-822-Z-99-001, 25 pp.
- U.S. Environmental Protection Agency. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th edition. U.S. Environmental Protection Agency. EPA 821/R-02-012, October, 2002. (Available online at <http://www.epa.gov/waterscience/WET/disk2/>; accessed October 2, 2005.)
- U.S. Environmental Protection Agency. 2004. USEPA Terminology Reference System, Last updated on Friday, March 26th, 2004 TRS Version 2003. <http://oaspub.epa.gov/trs>. Accessed on Friday, September 9th, 2005.
- Wildeman, T.R., Ranville, J. F., Herron, J., and Robinson, R. H. 2003. Development of a simple scheme to determine the chemical toxicity of mine wastes. p. 1501-1516. *In*: Proceedings of the 9th Billings Conference and 20th National Conference of the American Society for Surface Mining and Reclamation.
<https://doi.org/10.21000/JASMR03011501>